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13. ABSTRACT ***(Maximum 200 Words)***

E2A gene products (E47 and E12) are potent transcription factors containing the basic helix-loop-helix (bHLH) domain for DNA binding and dimerization. E2A proteins are thought to control cell growth and differentiation. E2A is also implicated to have a role as a tumor suppressor. E2A function can be inhibited by a group of inhibitors called Id. Thus, overexpression of Id proteins could diminish the function of E2A as a tumor suppressor, and potentially cause breast cancer. To test this hypothesis, we have generated transgenic mice expressing Id-1 in mammary epithelial cells, but have not detected any tumor formation. On the other hand, we have found Id-1 overexpression in a large fraction of archived human breast cancer samples. The significance of Id-1 overexpression in human breast cancer remains to be understood.

Furthermore, we have shown that E2A proteins in primary mammary epithelial cells and in the MDA-MB-231 breast cancer cell line are degraded through a proteasome mediated pathway. This provides an additional mode of regulation for E2A function in mammary epithelial cells.

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X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

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(4) TABLE OF CONTENTS

(5) INTRODUCTION

The E2A gene is ubiquitously expressed and encodes two alternatively spliced products, E12 and E47, which are potent transcription factors containing the basic helix-loop-helix (bHLH) domain for DNA binding and dimerization (Murre et al., 1989). E12 and E47 form homodimers or heterodimers with other tissue-specifically expressed bHLH transcription factors to bind to a consensus sequence called E box. These bHLH proteins have been shown to play crucial roles in the differentiation of a variety of cell types such as the lymphocytes, muscle cells, pancreatic cells, and neuronal cells (Zhuang et al., 1994; Bain et al., 1994; Weintraub. et al., 1991; Naya et al., 1995; Lee et al., 1995). In addition to dimerizing with the bHLH proteins and binding to DNA, E12 and E47 can also form heterodimers with the Id proteins (Id1-4), which has the HLH domain for dimerization but not the basic region for DNA binding. Therefore, E12 and E47 can be sequestered into inactive complexes and their function as transcription factors can be inhibited. Indeed, it has been shown that overexpression of Id proteins blocks the differentiation of many cell lineages including the B and T lymphoid, muscle, neuronal, adipose and mammary epithelial cells (Sun, 1994; Kim et al., 1999; Jen et al., 1992; Moldes et al., 1997; Desprez et al., 1995).

Apart from the differentiation function of E2A gene products, they have also been implicated to have a role as tumor suppressors. In NIH 3T3 fibroblasts, overexpression of E47 or incubation with Id antisense oligonucleotides arrests cell cycle at the G1 to S phase transition (Peverali, et al., 1994; Hara et al., 1994; Hara et al., 1994). We have shown that E2A can activate transcription of the gene encoding cyclin dependent kinase inhibitor, $p21^{CIP}$, through the E box sequences located in the promoter region of the p21 gene (Prabhu et al., 1997). In mice, disruption of the E2A gene (Bain et al., 1996; Yan et al., 1997) or overexpression of the Id-1 inhibitor in the T cell lineage (Kim et al., 1999) results in the development of T cell lymphoma at very high frequencies, thus suggesting a tumor suppressor function for the E2A proteins. Evidence implicating the tumor suppressor function of E2A has also come the studies of the TAL family of oncogenes (reviewed by Baer, 1993). The TAL proteins, including Tal1, Tal2 and Lyl1, are all bHLH proteins that can dimerize with E2A proteins and bind to E boxes. Aberrant expression of the Tal1 gene, for example, has been found in 70% of human acute lymphoblastic leukemia (T-ALL) samples. Although Tal1 can form heterodimers with E47 to bind to DNA, the heterodimers potentiate the transcription of their target genes very poorly as compared to E47 homodimers. We have shown that the poor transactivation by E47/Tal1 heterodimers is due to the incompatibility of the activation domains present in E47 and Tal1 (Park and Sun, 1998). We have then constructed a chimeric protein, E-T/2, which contains the N-terminus of E47 with the two known activation domains and the C-terminus of Tal1 including the bHLH domain for DNA binding and dimerization. The E-T/2 chimeric protein can activate transcription as heterodimers with E47 even more potently than E47 homodimers. To test the hypothesis that the normal function of E47 is inhibited in T-ALL cells as a result of aberrant Tal1 expression, we have introduced the E-T/2 construct into the Jurkat T-ALL cell line, in which the majority of E47 proteins are bound to Tal1 (Park et al., 1999). By competing

with Tal1 to bind to endogenous E47, E-T/2 was able to restore the transcription activity of E47. Because Tal1 does not form homodimers, E-T/2 would not form homodimers to activate transcription. The activity of E-T/2 is therefore limited to a near physiological level by the availability of endogenous E2A proteins. As a result of the restoration of E47 activity in Jurkat cells, the growth of Jurkat cells was dramatically inhibited, and apoptosis also occurred. These result would suggest that the mechanism by which Tal1 causes T cell leukemia might involves the inhibition of the tumor suppressing function of E2A proteins like E47. The principles and strategies of these experiments have been employed in our studies concerning breast cancer.

Regarding mammary epithelial cells, overexpression of Id-1 has been found to block the induced differentiation of a mouse mammary epithelial cell line, SCp2, which has been exposed to basement membrane and lactogenic hormones (Desprez et al., 1995). Moreover, these Id1 expressing SCp2 cells showed invasion of the basement membrane and resumption of cell proliferation. Recently, the same researchers have shown that Id-1 overexpression stimulates the expression of two novel polypeptides with gelatinase activities. It was therefore proposed that Id-1 expression may be related to the metastasis of breast cancer (Desprez et al., 1998). We have obtained preliminary evidence that Id-1 and Id-2 appear to be overexpressed in a fraction of breast cancer samples by using RT-PCR analyses. Based on these data, we have hypothesized that overexpression of Id proteins may lead to the inhibition of E2A function, which may then interfere with the normal process of mammary cell differentiation and tumor suppression. To test this hypothesis, we have generated transgenic mice expressing Id-1 in mammary glands to test if Id-1 expression leads to tumor formation. We have also examined Id-1 expression in human breast cancer samples using the in situ hybridization technique. We have then investigated the function of E2A proteins in mammary epithelial cells.

(6) BODY

A. Examination of MMTV-Id-1 transgenic mice

To test the effect of Id proteins on tumorigenesis and mammary gland development, we have generated transgenic mice carrying the Id-1 gene under the control of the mouse mammary tumor virus (MMTV) long terminal repeat (LTR). The Id1 cDNA was cloned into a modified MMTV transgenic vector obtained from Dr. B. Weinstein (Columbia University), which has been shown to direct high levels of expression in mammary tissues and low levels of expression in salivary gland and some male reproductive tissues (Yao and Weinstein, personal communication). We initially obtained six independent transgenic lines, of which two lines did not give any progenies, one line showed no expression, and three lines exhibited some levels of expression of the transgene upon preliminary analysis. Despite Id-1 expression detected in three lines of transgenic mice, no tumors were found in any of the transgenic mice after observation for up to 12 months. These transgenic mice have also been crossed with transgenic mice expressing the Her2/neu oncogene under the control of MMTV LTR. The resulting double transgenic mice did not develop any tumor. Therefore, we are tempted to conclude that Id-1 expression in our transgenic mice is not sufficient to cause breast cancer.

B. Detection of Id-1 overexpression in human breast cancer samples.

As a project planned to be carried out in Year 2, we proposed to examine human breast cancer samples for Id-1 overexpression by using the in situ RNA hybridization technique. The major challenge in this experiment is that in situ hybridization has to be carried out using archived and formalin fixed tissue imbedded in paraffin. We initially used dioxigenin-labeled antisense RNA probes for hybridization followed by chromogenic detection with anti-dioxigenin antibodies directly conjugated with alkaline phosphotase, or in combination with secondary antibodies conjugated with alkaline phosphotase. After many attempts, we found that the non-specific background as judged by the signals generated by using the sense probes on these archived and formalin fixed tissues is too high for the results to be reliable. We thus switched to ^{35}S-labeled RNA probes and the background was significantly reduced. We have used antisense β-actin probes as positive controls. A total of 26 breast carcinomas were analyzed for Id-1 overexpression. Eight samples are excluded due to poor RNA preservation demonstrated by very week signal of β-actin. Nine out of 18 breast carcinoma samples showed overexpression of Id-1. Representative samples with and without Id-1 overexpression are showed in Figure 6.

Table 1 summarizes the results of Id-1 expression along with clinical data. Id-1 overexpression is detected in 50% of human breast carcinoma samples, either ductal or lobular carcinoma. Id-1 overexpression is more frequently associated with younger patients (mean age = 49) compared to the mean age of patients with Id-1 negative carcinoma (mean age = 60). However, due to limited number of samples, the observed difference in mean age is not statistically significant.

Previous studies on human breast cancer cell line have suggested that Id-1 expression may suppress mammary epithelial cell differentiation and promote invasion (Desprez et al., 1995, Desprez et al., 1998). We have therefore evaluated the relationship between Id-1 overexpression and pathological grade of breast carcinoma or the rate of lymph node metastasis. Our results showed that carcinomas with or without Id-1 overexpression have very similar pathological grade (7.44 vs. 7.43) and lymph nodes metastasis rate (83% vs. 86%).

In NIH 3T3 fibroblasts, overexpression of E47 or incubation with Id antisense oligonucleotides arrests cell cycle at the G1 to S phase transition (Peverali, et al., 1994; Hara et al., 1994; Hara et al., 1994). We thus attempted to determine whether Id-1 overexpression was associated breast carcinoma proliferation. We examined the relationship between Id-1 overexpression and mitotic activity of human breast carcinoma, and found no significant difference in mitotic figures between carcinomas with (mean = 9.2) and without (mean = 8) Id-1 expression.

Taken together, we have demonstrated that Id-1 is overexpressed in 50% of human breast carcinoma samples. However, Id-1 overexpression is not associated with generally applied clinical prognostic parameters such as pathological grade, lymph node metastasis, tumor size, and mitotic activity. It remains to be further investigated whether Id-1 overexpression influences the induction or progression of human breast carcinoma or whether it can be used as a molecular parameter for prognosis.

C. Examination of E2A protein turnover in MDA-231 breast cancer cells.

Results from our previous studies have suggested that the amount of E2A proteins may be regulated at the level of degradation. We found that this is particular the case in the estrogen-independent breast cancer cell line, MDA-MB-231. As shown in Fig. 2A, although E2A proteins are expressed at a level similar to that in WEHI231 B cells, they predominantly exist as a degraded form, which lacks the C-terminus. In T47D estrogen dependent breast cancer cell line, the level of E2A proteins is low than that in MDA-231 cells but the extent of degradation is also less. In addition, E2A proteins in mouse primary mammary epithelial cells before and after a 4-day culture are also present as the degraded form (Fig. 2B and C). This degraded form of E2A proteins is largely cytoplasmic as determined either by cell fractionation (Fig. 2B) or by immunohistochemistry (Fig. 3). As a result, no DNA binding complexes were detectable in mammary epithelial or breast cancer cells (Fig. 4).

To determine if the degradation of E2A proteins is mediated through proteasomes, specific inhibitors, MG-132 and lactacystin, were added to MDA-231 and T47D cultures for 2 or 4 hours. As shown in Fig. 5, MG-132 significantly blocked the accumulation of degraded E2A while lactacystin had some effect in the first two-hours. Perhaps, lactacystin is a labile inhibitor and may become ineffective over a longer period of incubation. In contrast, neither a non-specific protease inhibitor (leupeptin) nor the vehicle control (DMSO) had any effect on E2A degradation.

In summary, these results suggest that in mammary epithelial cells, E2A proteins, though expressed at a high level, are inactivated through mechanisms such as proteasome-mediated degradation. The biological significance of this novel regulatory mode of E2A function remains to be understood. It is also not clear whether E2A is constitutively inactivated or it functions to suppress excessive cell proliferation in a temporal manner when the need arises. If the latter is true, loss of E2A function under certain circumstances could potentially lead to breast cancer.

(7) KEY RESEARCH ACCOMPLISHMENTS

- Id-1 transgenic mice generated but no tumor formation was found in these mice.
- Id-1 overexpression detected in 50% of human breast cancer samples.
- E2A proteins found to be rapidly degraded through a proteasome-mediated pathway in primary mammary epithelial cells and breast cancer cell line, MDA-MB-231.

(8) REPORTABLE OUTCOMES

- Manuscripts, abstracts presentations: Abstract for a poster at DoD Breast Cancer Program "Era of Hope" meeting, Atlanta, GA 2000.
- patents and license applied for and/or issued: None.
- degrees obtained that are supported by this award: None.
- development of cell lines, tissue or serum repositories:
  Id-1 transgenic mouse lines, pYY-Id1-30 and pYY-Id1-59.
- informatics such as databases and animal models, etc.
- funding applied for based on work supported by this award: None

- employment or research opportunities applied for and/or received on experiences/training supported by this award: None.

(9) CONCLUSIONS

Studies in Year 2 of the proposal have discovery Id-1 overexpression in a large fraction of human breast cancer. A novel mechanisms of E2A protein degradation have also be found in mammary epithelial cells. Both of these findings may suggest means to inactivate the E2A transcription factor and tumor suppressor, which could affect breast cancer development.

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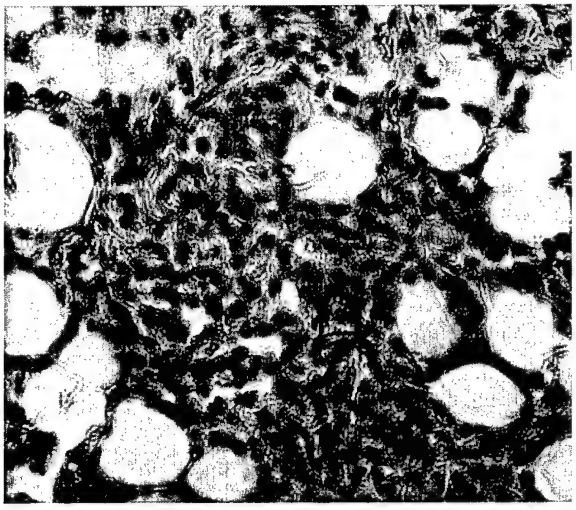
(11) APPENDICES:

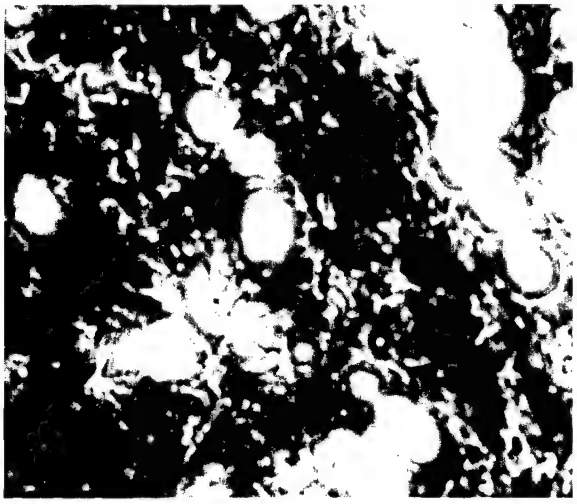
(a) Figures 1-5.

(b) CV of the PI.

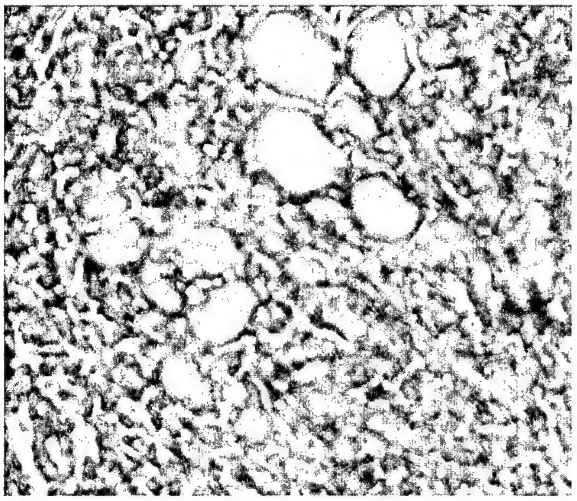
Figure 1A Breast Carcinoma which overexpress Id-1



H&E section human breast carcinoma. (10x)

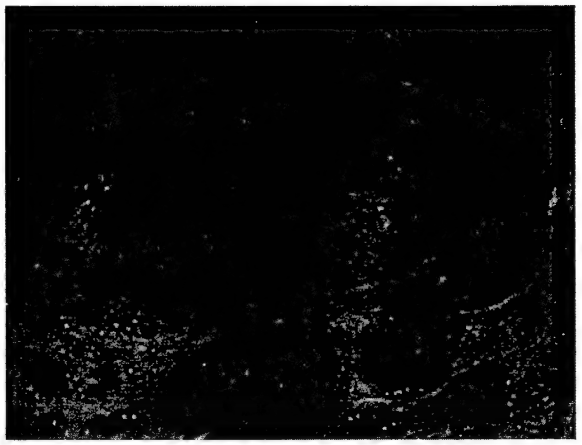


In situ hybridization with Id-1 antisense probe. (10x)

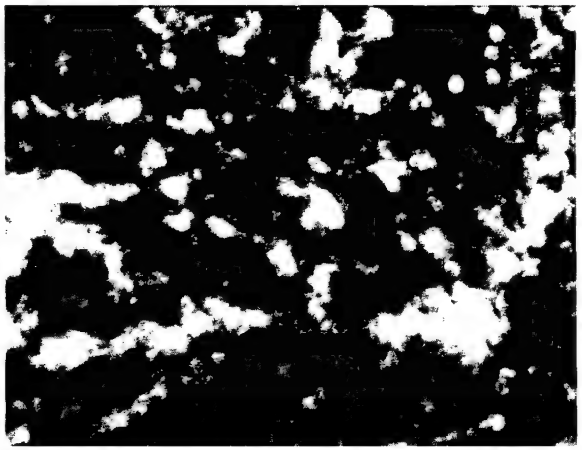


In situ hybridization with Id-1 sense probe. (10x)

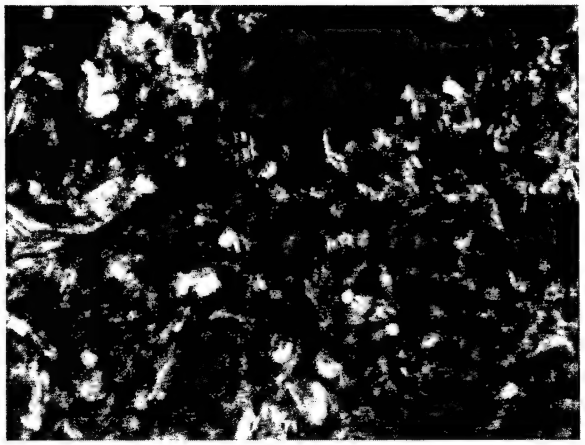
Figure 1B. Breast Carcinoma which does not overexpress Id-1



H&E section of breast carcinoma 10x

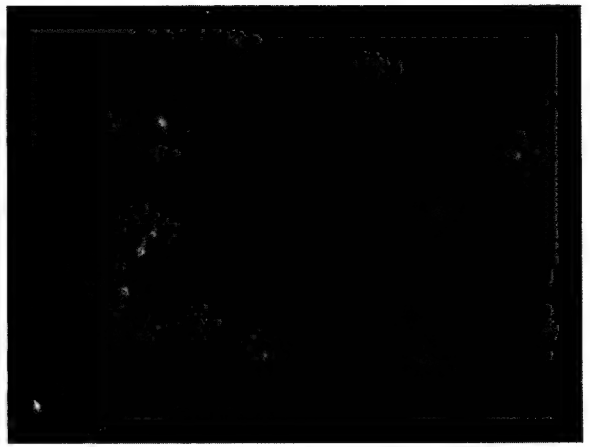


In situ hybridization with ß-Actin antisense probe



In situ hybridization with ß-Actin sense probe

In situ hybridization with Id-1antisense probe



In situ hybridization with Id-1sense probe

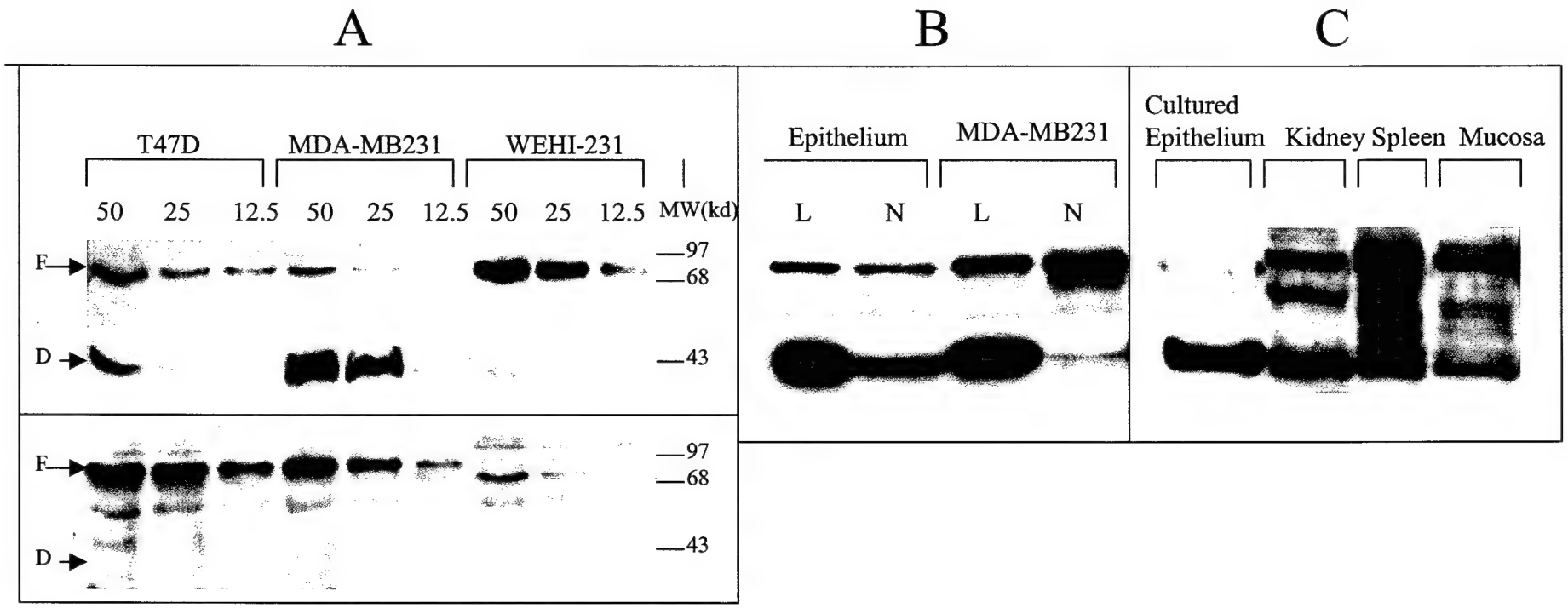


Figure 2. Western blotting analyses of E2A proteins protein. (A) Whole cell lysates from T47D, and MDA-MB231 breast cancer cell lines as well as WEHI-231 lymphoid cell lines were analyzed. The different amounts of proteins (μg) were loaded as indicated on the top of the lanes. Two forms of E47 protein, full length (F) and degraded (D) forms labeled with arrows were detected with antibodies against the N-terminus of E2A proteins (the bottom). Because WEHI231 is a mouse cell line, the antibodies generated using human E47 do not cross react with the mouse E2A proteins. (B) The whole cell lysate (L) and nuclear extract (N) were prepared from purified 14-week mouse mammary epithelial cells and MDA-231 cells. Western blot was carried out with antibodies against the N-terminus of E2A proteins. (C) Nuclear extracts were prepared from normal mouse kidney, spleen, mucosa and purified mammary epithelial cells cultured for 4 days. Western blot was carried out with antibodies against the N-terminus of E2A proteins.

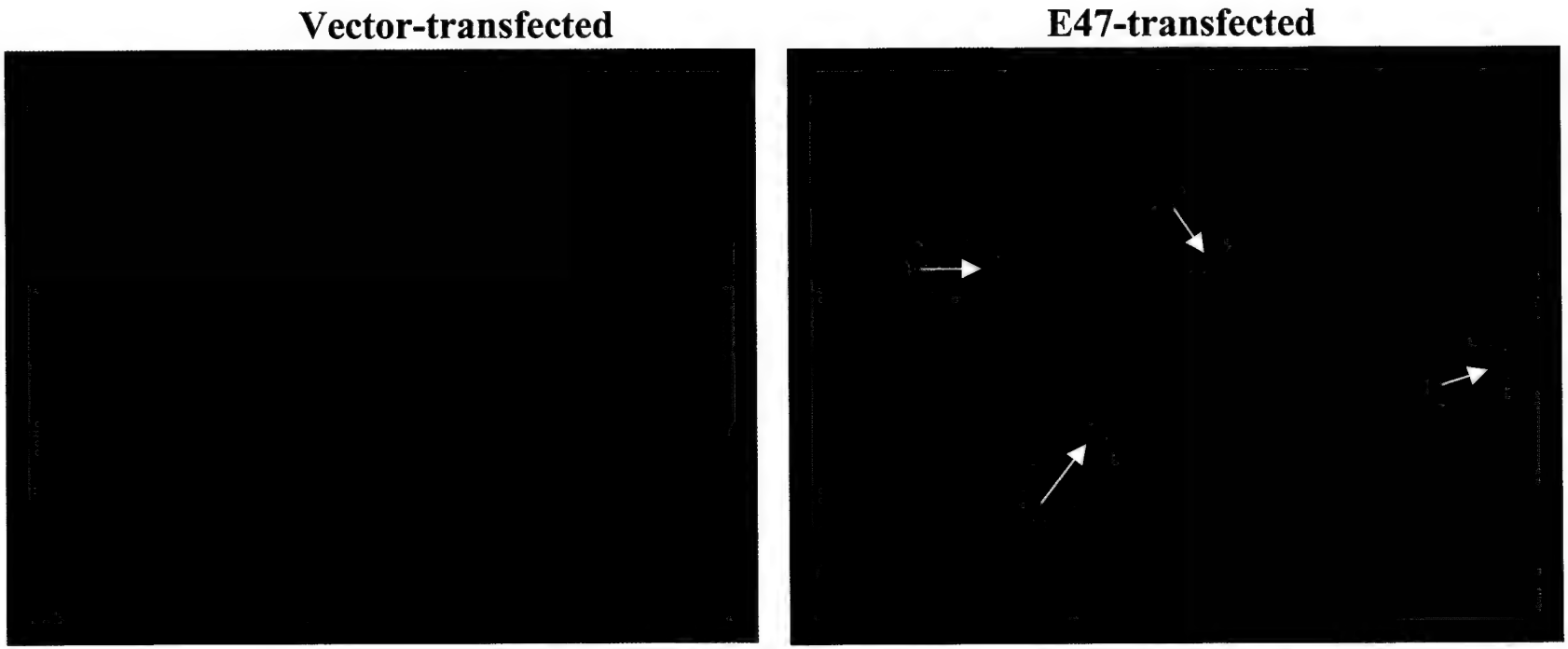


Figure 3. Immunohistochemistry of MDC-MB-231cells transfected with pcDNA3 vector or pcDNA3-E47. Forty-eight hours after transfection, The cells were fixed with 4% paraformaldehyde, and permeablized with methanol at –20C°. The cells were then blocked with 10% FCS. The E47 protein was visualized using polyclonal antibody against the N-terminus of E47 and rhodamine-conjugated secondary antibody. Cells overexpressing E47 after transfection are indicated by arrows.

Figure 4. Electrophoretic mobility shift assay.

Nuclear extracts isolated from the indicated cell lines were used in a binding reaction with ^{32}P-labeled E-box-containing probe. Antibodies against E47 was added to the reaction in Lane 2. The arrow indicates the E47 homodimer binding complex.

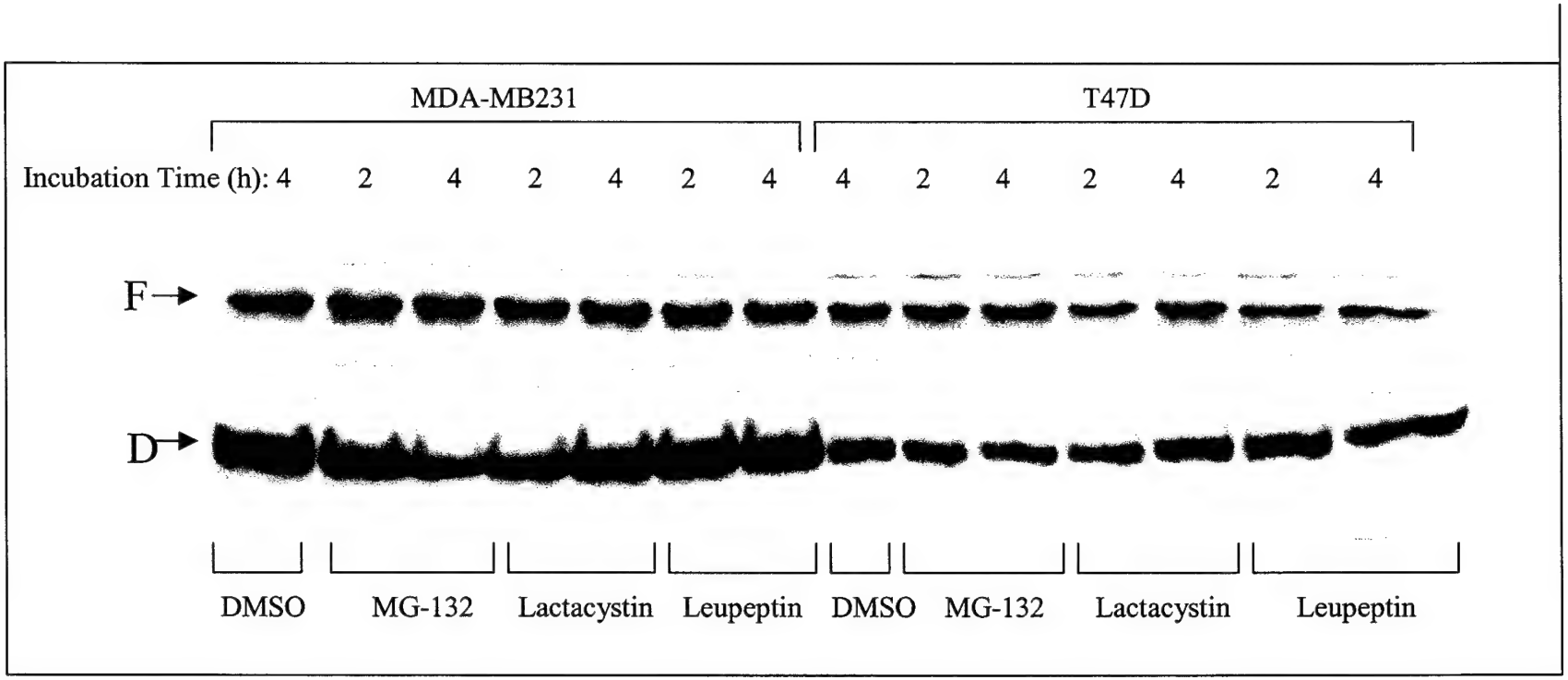


Figure 5. Proteasome-mediated degradation of E2A proteins.

MDA-MB231 and T47D cells were incubated in the presence of 2.5 μM of proteasome specific inhibitor, MG-132 or lactacystin, protease inhibitor, leupeptin, and DMSO for 2 and 4 hours. Western blot analysis was carried out with anitbodies against the N-terminus of E2A proteins. Full-length and degraded forms of the proteins are as labeled.

Table 1. Id-1 expression and clinical data of human breast carcinomas

Sample	Id1	Age	tumor type	Grade	Size	LN	Mitosis
98-59	-	87	d	8	2.3		4
98-4575	-	62	l		2.4	y	2
98-11562	-	53	d	5	1.3	n	5
98-11933	-	41	l		6.5	y	12
98-12755	-	56	d	7	3	y	3
98-12037	-	46	d	8	2.2	y	15
98-17298	-	48	d	7	10	y	3
99-517	-	75	d	9	1.6	y	20
99-726	-	69	d	8	1.5	y	8
Average		59.7		7.4	2.7		9
98-3800	+	49	d	8	1.6		6
98-9336	+	47	d	5	6	y	2
98-14396	+	48	d	7	10	y	3
98-14936	+	38	d/l	9	2	y	34
98-20976	+	53	d	6.5	3.5		4
99-760	+	56	d	8	2.5	y	9
99-1336	+	33	l	6.5	2.2		3
99-1769	+	76	d	8	1.2	y	17
99-4279	+	52	d	7	2.1	n	2
Average		49		7.4	3.4		8

D = ductal; l = locular; LN = lymph node metastasis; size is in cm.

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Curriculum Vitae

EDUCATION:

1978 - 1981	Extramural student, in Basic Medical Sciences, Beijing Medical University, China.
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1987 - 1988	Whitehead Institute for Biomedical Research, Mentor: Dr. David Baltimore, Field: protein synthesis inhibition by poliovirus and anti-HIV strategies.
1989 - 1991	Whitehead Institute for Biomedical Research, Mentor: Dr. David Baltimore, Field: structure and function of basic helix-loop-helix transcription factors.

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1991 - 1998	Assistant Professor Department of Cell Biology, New York University School of Medicine.
1998 - 1999	Associate Professor Department of Cell Biology, New York University School of Medicine.
1999 – present	Associate Member Immunobiology & Cancer Research Program Oklahoma Medical Research Foundation.

OTHER PROFESSIONAL ACTIVITIES:

1991 - 1998	Reviewer for "Molecular and Cellular Biology", "Nucleic Acids Research", ""Journal of Virology", Journal of Cellular Physiology", Journal of Neuroscience" and 'Journal of Immunological Methods".
1993	Reviewer for grants received by the United States Army Medical Research and Development Command for research on breast cancer.
1994 - 1998	Seminar speaker at NIA, Thomas Jefferson Cancer Center, Harvard Medical School, Yale Medical School, NCI, UCLA and UMDNJ.
1995	Invited speaker at the Sixth International Symposium of the Society of

	Chinese Bioscientists in America, Vancouver, Canada.
1995 -1997	Reviewer and site-visitor for two NIH (NIDDK) program project grants.
1996	Speaker at the Joint Symposium of American Association of Immunologists, American Association of Biochemists and American Associate of Investigative Pathologists, New Orleans, LA.
1997	Invited speaker at the Seventh International Symposium of the Society of Chinese Bioscientists in America, Toronto, Canada
1998	Invited speaker at Gene Therapy/Molecular Biology International Conference, Heraklion island of Crete, Greece.
1998	Invited speaker at New England Immunology Conference, Woods Hole, MA.
1999	Invited speaker at Gene Therapy/Molecular Biology International Conference, Redwood City, CA.
1999	Session chair and speaker at the Eighth International Symposium of the Society of Chinese Bioscientists in America, Hong Kong.

PROFESSIONAL SOCIETIES:

1991	Member of the American Association for the Advancement of Science.
1992	Member of the American Society for Microbiology.
1999	Member of the American Association of Immunologists.
1994	Member of the Society of Chinese Bioscientists in America. Served in the Council (1996) and the Membership Committee (1994-1996) of Society of Chinese Bioscientists in America. President of the Tri-State Chapter (1997-1998).
1998	Member of Ray Wu Society for Life Sciences. Board of the Directors (1998-2000).

AWARDS:

1981	CUSBEA (China-United States Biochemistry Examination and Application) exchange student.
1989 - 1991	Cancer Research Institute Postdoctoral Fellowship.
1991 - 1996	Markey Scholar (Supported by a grant from The Lucille P. Markey Charitable Trust Foundation to NYUSM).
1992 - 1993	Whitehead Presidential Fellowship.
1992 - 1996	Cancer Research Institute Investigator Award.
1994 - 1998	Irma T. Hirschl Trust Career Scientist Award.

PAST MAJOR GRANTS:

1991 - 1997	The Lucille P. Markey Charitable Trust Foundation. "Molecular mechanisms of Hematopoiesis" Total direct cost $511,875.
1992 - 1994	American Cancer Society Research Grant. "Regulation of the Id genes in B cell development". Total direct cost $146,380.
1992 - 1997	Cancer Research Institute Investigator Award. "Role of the HLH Id proteins in B lymphoid differentiation and neoplasia". Total direct cost $200,000.
1994 - 1998	Irma T. Hirschl Trust Career Scientist Award. "Molecular mechanisms of B cell differentiation". Total direct cost $100,000.
1994 - 1999	NIH R01 Grant from NIAID Regulation of the Id genes in B cell development. Total direct cost $397,046.

ACTIVE GRANTS

1998 - 2001	US Army "The role of Id proteins in breast cancer." Total direct cost $210,000.
1998 - 2001	American Cancer Society The molecular mechanism of T cell leukemogenesis induced by TAL. Total direct cost $300,000 (returned).
1998 - 2003	NIH R01 Grant from NCI. The molecular mechanism of T cell leukemogenesis induced by TAL. Total direct cost $915,144.
1999 - 2000	NIH R21 grant from NIAID Regulation of the Id genes in lymphocyte development. Total direct cost $107,381

TEACHING EXPERIENCE:

Graduate and Medical School Courses:

1992	Nucleic Acids Core Course for graduate students.
1992	Molecular and Cellular Biology
1994 - 1999	Cell Biology of Tissues and Organs (Histology).

Graduate School Training:

1991 - 1999 New York University School of Medicine.

Thesis advisor: Four graduate students and one MD-PhD student. (three students received Ph.D.).
Thesis committee member: Five graduate students and one MD-PhD student.

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1. Tso, J. Y., **Sun, X.-H.,** Kao, T.-h., Reece, K., and Wu, R. (1985) Isolation and characterization of rat and human glyceraldehyde 3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. *Nucl. Acids Res.* 13: 2485-2502.

2. Tso, J. Y., **Sun, X.-H.** and Wu, R. (1985) Structure of two unlinked *Drosophila melanogaster* glyceraldehyde 3-phosphate dehydrogenase genes. *J. Biol. Chem.* 260: 8220-8228.

3. **Sun, X.-H.,** Lis, J. and Wu, R. (1988) The positive and negative transcriptional regulation of the *Drosophila Gapdh*-2 gene. *Genes Dev.* 2: 743-753.

4. **Sun, X.-H.**, Tso, J. Y., Lis, J. and Wu, R. (1988) Differential regulation of the two glyceraldehyde-3-phosphate dehydrogenase genes during *Drosophila* development. *Mol. Cell. Biol.* 8: 5200-5205.

5. **Sun, X.-H.**, and Baltimore, D. (1989) Human immunodeficiency virus tat-activated expression of poliovirus protein 2A inhibits mRNA translation. *Proc. Natl. Acad. Sci. USA* 86: 2143-2146.

6. Kamps, M. P., Murre, C., **Sun, X.-H.**, and Baltimore D. (1990) A new homeobox gene contributes the DNA-binding domain of the t(1:19) translocation protein in pre-B ALL. *Cell* 60: 547-555.

7. **Sun, X.-H.** and Baltimore, D. (1991) An inhibitory domain of E12 transcription factor prevents DNA binding in E12 homodimers but not in E12 heterodimers. *Cell* 64: 459-470.

8. **Sun, X.-H.**, Copeland, G., Jenkins, N. and Baltimore, D. (1991) The Id proteins Id1 and Id2, selectively inhibit DNA binding by one class of helix-loop-helix proteins. *Mol. Cell. Biol.* 11: 5603-5611.

9. **Sun, X.-H.** (1994) Constitutive expression of the Id1 gene impairs mouse B cell development. *Cell* 79: 893-900.

10. Saisanit, S. and **Sun, X.-H.** (1995) A novel enhancer, the pro-B enhancer, regulates Id1 gene expression in progenitor B-cells. *Mol. Cell. Biol.* 15: 1513-1521.

11. Laure, T. M., Starovasnik, M. A., Weintraub, H., **Sun, X.-H.** and Klevit, R. E. (1995) MyoD forms micelles which can dissociate to form heterodimers with E47. Implications of micellization on function. *Proc. Natl. Acad. Sci. USA.* 92: 11824-11828.

12. Mahajan, M. A., Park, S. T., and **Sun, X.-H.** (1996) Association of a novel GTP-binding protein, DRG, with TAL oncogenic proteins. *Oncogene* 12: 2343-2350.

13. Vitola, S. J., Wang, A. and **Sun, X.-H**. (1996) Substitution of basic amino acids in the basic region stabilizes DNA binding by E12 homodimers. *Nucleic Acids Res.* 24:1921-1927.

14. Saisanit, S. and **Sun, X.-H**. (1997). Regulation of the pro-B-cell-specific enhancer of the Id1 gene involves the C/EBP family of proteins. *Mol. Cell Biol.* 17: 844-850.

15. Chen, B., Han, B. H., **Sun, X.-H.** and Lim, R. W. (1997). Inhibition of muscle-specific gene expression Id3: requirement of the C-terminal region of the protein for stable expression and function. *Nucl. Acid. Res.* 25: 423-430.

16. Prabhu, S., Ignatova, A., Park, S. T. and **Sun, X.-H**. (1997). Regulation of the expression of cyclin-dependent kinase inhibitor p21 by E2A and Id proteins. *Mol. Cell. Biol.* 17: 5888-5896.

17. Dang, W., **Sun, X.-H**. and Sen, R. (1998). ETS mediated cooperation between basic helix-loop-helix motifs of the immunoglobulin mu heavy chain gene enhancer. *Mol. Cell. Biol.* 18: 1477-1488.

18. Park, S. T. and **Sun, X.-H**. (1998). The Tal1 Oncoprotein inhibits E47-mediated transcription: Mechanism of Inhibition. *J. Biol. Chem.* 273: 7030-7037.

19. Park, S. T., Nolan, G. P. and **Sun, X.-H.** (1999). Growth inhibition and apoptosis due to restoration of E2A activity in T cell acute lymphoblastic leukemia cells. *J. Exp. Med.*,189: 501-508.

20. Pan, L., Sato, S., Frederick, J. P., **Sun, X.-H.** and Zhuang, Y. (1999) Impaired immune responses and B cell proliferation in mice lacking the Id3 gene. *Mol. Cell. Biol.* 19: 5969-5980.

21. Kim, D., Peng, X., and **Sun, X.-H.** (1999). Massive apoptosis of thymocytes in T cell deficient Id1 transgenic mice. 19: 8240-8253.